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Application No. S61-246844, filed October 17, 1986; Inventors: Tadako OGURO, Chiaki SANO, Shigemitsu ABE, Masazumi DATE, Tetsuya KAWAKITO; Assignee: Ajinomoto Co. Inc.

PRODUCTION OF L-HISTIDINE FROM BONITO BROTH

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1. Title of Invention:

Production of L-Histidine from Bonito Broth

2. Claims

A method of recovering L-histidine from bonito broth characterized in that in the process of producing shaved bonito, the pH of the bonito broth that is produced during the thorough boiling of bonito is adjusted to 1 ~ 5 or 2 ~ 4 as desired by adding hydrochloric acid, the liquid is heated, and the water layer that contains oil component in the top layer and denatured protein in the bottom layer is removed by layer separation, whereupon a high polymer fraction is removed by a semi-permeable membrane, concentrated and cooled recovering L-histidine as the L-histidine hydrochloride-monohydrate.

3. Detailed Explanation of Invention

(Field of Applicability in Industry)

This invention concerns a method of producing L-histidine whereby L-histidine from the bonito broth that is a by-product of the shaved bonito production process, is recovered in the form of L-histidine hydrochloride-monohydrate.

(Purpose of Invention)

The purpose of this invention is to offer a technology to separate and purify cheaply L-histidine in the form of L-histidine hydrochloride monohydrate from bonito broth that had no use other than an extract in Prior Art.

(Prior Art)

The bonito broth which is a byproduct of the shaved bonito production is known to normally contain natural amino acids in the amount of 10 – 15% of its solids; of these L-histidine (abbreviated to L-His) accounts for about 80%. However, apart from amino acids, this broth also contains large amounts of other components, such as proteins, inorganic salts, fats, peptides, etc., therefore so far the amino acids have not been used in particular as raw materials in L-His production, but rather the broth was partially concentrated and sold as bonito extract, while the rest was thrown away.

On the other hand, there are reports that an advantage of membrane separation for aquaculture is its application for the production of Fish Soluble") (Oji Nomura "The Newest Membrane Processing Technology and Its Application", p. 639-642 (1985), published by Fuji Techno System Corp.); sardines, mackerels, and other coastal fishes, as

well as krill are used as the raw material. Fish Soluble has a low His content of 5.8% among amino acids, and the conclusion is that the acrylonitrile GM-80 manufactured by Amicon Co. is a good ultrafiltration membrane, but the acrylonitrile synthetic membrane is deficient in alkali resistance, heat resistance (<<Ultrafiltration System>> No. S 82-01 – 1000 from the Asahi Kasei Corp. Catalogue) and is hard to apply to this system because it is hard to sterilize and rinse chemicals from.

(Problems to be Solved by this Invention)

(a) As indicated above, bonito broth contains natural His in the amount of 8 – 12% of its solids, but since its moisture content is 90% or more, in order to separate His by crystallization, concentration by a factor of at least 10 is required. However, due to concentration the viscosity of bonito broth is dramatically raised, and since even at normal temperature it has gelling properties, it is hard to obtain His from bonito broth by concentration and crystallization as is.

(b) Since bonito broth contains inorganic salts in an amount that is about 7 times the amount of His in ion equivalent, methods that rely on ion adsorption and desorption by ion exchange resin, that normally serve well in the purification of amino acids, are not practicable because due to the extremely low amount of His adsorbed by the ion exchange resin, the cost of ion exchange resin will become high.

On the other hand, as a method of separating the large quantities of inorganic salts and His, consideration has been given to electrodialysis in the vicinity of isoelectric point (pH 7.5) where His does not have an electric charge (C. J. Cox et al., J. Biol. CHem, 81, 755 (1929)). When the pH of the broth is elevated to around 7.5 by adding alkalis, crystals with extremely poor filtering properties precipitate that are considered to be crystals of hydrated sodium calcium pyrophosphate. Even if these crystals are separated by filtering, still a lot of electric power is required for the electrodialysis of the large amount of inorganic salts, so this method is deficient because it is impractical.

As described above, it is characteristic of bonito broth that its viscosity rises in the course of concentration and the broth solidifies and, in addition, it contains a large amount of inorganic salts; therefore it is hard to separate His by crystallization simply by concentrating the broth; furthermore, purification by treatment with an ion exchange resin and desalination by means of electrodialysis are deficient in their impracticality because the purification cost is too high. Therefore, the recovery of His contained in the broth requires solving these problems.

(Means of Solving the Problems)

The authors of this invention conducted focused research to solve these problems; they first elevated the viscosity of the bonito broth in the course of concentration and investigated the component that solidifies it into jelly form; they identified it as a high molecular compound whose core is a protein that is present in the broth in a dissolved form. Then discovered that the use of a semi-permeable membrane that has a high-molecule fractioning property constitutes an extremely effective method of removing this high molecular protein.

A suitable semi-permeable membrane used here would have molecular fractioning properties in an ultrafiltering zone, and the fractioned molecular weight would be in

several dozen thousands or less, preferably, in several thousand. Moreover, the regular membrane material such as polyacrylonitrile, polysulfone, polyolefin etc. can be used, but as a result of various investigations, polysulfones are easier to used for this purpose from the standpoint of heat resistance characteristics and resistance to chemicals (in particular, to alkalis) in washing. Regular types of filter can be used, such as tubular, flat, flow filter, etc, but since usually the broth includes insoluble components derived from the broth such as dirt, etc., to extend the service life of the filter, it is useful to minimize the admixture of such foreign matter that can easily cause clogging of the filter mesh by providing a 100 ~ 200 mesh front filter.

The broth itself contains a lot of nutritional components, and readily goes bad, therefore reducing the typical broth pH of 6 ~ 7 to pH 1 ~5 or, preferably, 2 ~ 4, by adding hydrochloric acid or other acids and thermal sterilization to prevent the proliferation of germs in the broth and prevent the decomposition of His are extremely effective. The time required for thermal sterilization depends on temperature but in the experience of the authors of this invention it took about 1 hr at 70°C. The heating temperature is within such a range that His decomposition losses or racemization will not occur. Heating at this acidity has been found to be effective in making the soluble protein in the broth denature and precipitate, when the heated up broth is left to sit and making the fat float up. Upon static fractioning, due to separation, while preventing the top layer (fat) and the bottom layer (water layer containing denatured protein) from separately mixing with a middle layer (a water layer containing His), the amount of solids other than fat and His in the broth drops, thus it is possible to reduce the filtration load in the subsequent process of filtering by means of a semipermeable membrane and accelerate the filtering speed. If instead of static fractioning or in combination with it, the heated broth is subjected to centrifugal separation, the advantage is that bottom layer is reduced while the water layer that contains His, which is the middle layer, is increased. In an experimental case of this invention, no loss of His to decomposition was confirmed to have been caused by heating (70°C, 1 hour) in a weakly acidic zone (pH 2 ~ 4).

The broth contains large amounts of inorganic salts and this makes it difficult to purify His. However, as a result of their research the authors of this research found that, surprisingly, the impacts of inorganic salts on the crystallization of His and L-histidine hydrochloride-monohydrate (His · HCl · H_2O) are directly opposite in the pure system and the broth system. That is, the effect of inorganic salts on the crystallization His · HCl · H_2O at pH 3.0 and on His at pH 7.5 (free) was as follows. In the zone where His · HCl · H_2O was present at pH3.0 the solubility of His · HCl · H_2O at 10°C was 9.29 g /dl in terms of His (at the bottom of the liquid: His · HCl · H_2O) in a pure system, whereas in the broth system it was 2.6 g/dl (at the bottom of the liquid: His · HCl · H_2O) which is significantly lower, demonstrating the effect of salting-out. However, in the zone where His was present at pH 7.5 the solubility of His at 10°C in a pure system was 3.1 g/dl (at the bottom of the liquid : His) while in the broth system it was 6.2 g/dl (at the bottom of the liquid : His) demonstrating the effect of salting-in, and demonstrating results that were the direct opposite of those for the pure system. It became clear that even in a system that contains a lot of impurities, such as that of the broth, His · HCl · H_2O can yield large crystals with good separability whereas His (free) is to a large extent affected by impurities in the broth, becoming minute and hard to separate.

As described above, with regard to the two tasks mentioned above by means of 1) removing a high-molecular substance with a semi-permeable membrane and 2) using the large amounts of inorganic salts as the salting-out agent in the crystallization of His · HCl · H_2O, a method has been established to achieve low solubility without crystal growth being affected adversely and retrieve His from bonito broth as large crystals of His · HCl · H_2O.

Below, we will explain this invention further through a Practical Examples and Comparison Examples.

Practical Example 1

200 l bonito broth had its pH reduced to 3.0 with hydrochloric acid and was heated to 70°C; its temperature was then maintained for 1 hour; the oil of the top layer and the water layer containing denatured protein of the bottom were removed and the water layer containing His (middle layer) was superfiltered by passing through a membrane (DDS Co., GR-81-PP (flat membrane), 2.25 m2, fractioning molecular weight 6000); diafiltration was performed while adding 20 l water and from the 200 l liquid that was passed 20 l high molecular solution was obtained. This resulting liquid was condensed to a His concentration of 8 dg / dl and then crystallized by cooling to 10°C; 1000 g His · HCl · H_2O was obtained.

The crystal purity of the crystals was 95% or more. The crystals were dissolved again and, upon decoloration with activated charcoal, recrystallized, yielding a product that met the standard for "L-histidine hydrochloride" in the Japanese Standards for Pharmaceutical Ingredients.

Comparison Example 1

200 l bonito broth was processed as in the Practical Example 1 except that it was not superfiltered; when it was condensed to a His concentration of 8 dg/dl and cooled to 10°C, the liquid's viscosity increased and it solidified as it cooled down; no His · HCl · H_2O precipitation was observed.

Comparison Example 2

200 l bonito broth was processed as in the Practical Example 1 up to the superfiltration processing, and 200 l filtered liquid was obtained. When caustic soda was added to this filtered liquid to raise its pH to 7.5 large amounts of crystals of hydrated sodium calcium pyrophosphate were crystallized; they were very hard to separate. These crystals were separated with a precoated cutting filter and condensed to 8 g /dl in terms of His, whereupon crystallization was performed by cooling to 10°C. However, as mentioned above, solubility increased and only 500 g His crystals was obtained. Moreover, these crystals were extremely fine, with poor separability, and contained 30 ~ 50% adsorbed mother liquor.

These His crystals were dissolved again and by adding hydrochloric acid the pH was brought to 3 in a His · HCl · H_2O zone; upon decoloration with activated charcoal and recrystallization purified His · HCl · H_2O crystals were obtained, but the crystals retained a tea-colored hue and did not meet the standard for "L-histidine hydrochloride" in the Japanese Standards for Pharmaceutical Ingredients.

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